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(54) Title: IMPROVED METHOD FOR DETECTION OF SPECIFIC TARGET CELLS IN SPECIALIZED OR MIXED
CELL POPULATION AND SOLUTIONS CONTAINING MIXED CELL POPULATIONS

(57) Abstract

  The invention relates to a method for detecting specific target-cells in a simple and time saving way, using paramagnetic
particles, antibodies recognizing the Fc portions of target-cell associating antibodies and target-cell associating antibodies direct-
ed to specific antigen determinants in the target-cell membranes. Incubation of the cell suspension with a mild detergent and/or
second set of antibodies or antibody fragments, prelabeled or not with fluorescent agents, metallocolloids, radioisotopes, biotin-
complexes or certain enzymes allowing visualization, will dramatically increase the specificity of the method. The method can fur-
ther be used for isolation of the target-cells by magnetic field application and a kit for performing the method according to the
invention is described.

1

Improved method for detection of specific target cells in
specialized or mixed cell population and solutions containing
mixed cell populations


The present invention relates to an immunomagnetic method for
detection of specific target cells in cell populations and
solutions of cell populations. The invention also relates to a
kit for performing the method in different cell populations.


In biology, biochemistry and adjacent fields it is considerable
need for methods in which chemical entities are linked
together. Such methods have an increasing importance in
research regarding both normal and abnormal cell populations.
Especially when solid supports are used cells can be
immobilized, detected and isolated and purified. Furthermore,
the cell content may be examined using a range of sofisticated
methods. It is also of importance to be able to isolate the
cells in viable forms.


Affinity binding is a sofisticated way of linking chemical/bio-
chemical entities together. In such a method a pair of binding
partners, which for example are attached to the substances to
be linked, bind to each other when brought in contact. One of
the binding partners in such a linkage may be represented by a
molecule on the cell surface. Several such binding partner
systems are known, such as antigen- antibody, enzyme- receptor,
ligand- receptor interactions on cells and biotin- avidin
binding, of which antigen-antibody binding is most frequently
used. A hapten/anti-hapten binding pair method has also
recently been suggested (PCT/EP90/01171).


When such methods are used for isolation of specific cells,
which are the subject for further various examinations, it is
necessary to reverse the linkage without producing destructive
effects on the binding partners, which ideally should recover
their function upon returning to the original conditions. This
is not always the case, although it is proposed a method for

adequately cleaving antigen/anti-antigen and hapten/anti-hapten
linkages (PCT/EP91/00671, PCT/EP90/01171).

Methods are known in which one of the binding partners is
attached to an insoluble support, such as paramagnetic
particles, and by which isolation of target cells in a mixed
cell population is performed as negative isolation or positive
isolation. In a negative isolation procedure the unwanted cells
can be removed from the cell preparation by incubating the
cells with antibody-coated particles, specific for the unwanted
cells. Following the incubation the cell/antibody/particle-
complex can be removed using the particles, leaving the wanted
target cells behind. This result is often not satisfactory,
since the wanted cells are left in the cell population, more or
less purified, and also since the intention of the isolation
procedure is to examine and/or perform further studies on the
specific target cells. Attempts have been made to use particles
for positive isolation, in which the wanted target cells are
removed from the mixed cell population. These procedures have,
however, been directed to certain target cells and are not
suited for all target cell systems. A positive isolation
procedure involving the hapten/anti-hapten linkage system has
recently been proposed (PCT/EP90/01171) and also a method for
isolating haemopoietic progenitor cells from bone marrow
(PCT/EP90/02327). The latter is directed to use a combination
of positive and negative selection for the purpose of isolating
and possibly growing specific cells, i.e. haematopoietic
progenitor cells, in the bone marrow, and is dependent upon
removal of the particles.

PCT/EP90/01171 relates to a method of connecting target cells
to an insoluble support by using the abilities of hapten, anti-
hapten antibodies and anti-cell antibodies to bind to each
other, thus constructing a linkage between the insoluble
support, i.e. particle, and the target cell, consisting at
least of hapten and anti-hapten antibody or combinations of
hapten and anti-hapten antibodies and anti-anti-hapten
antibodies or secondary anti-cell antibodies. The later

cleavage of the complex is performed by again exposing it to
hapten or hapten analogue. Thus the constructed link always
consists of hapten in addition to 1 or more elements. The
method is directed to unspecified target cells and is directed
to isolation of target cells and release of the insoluble
support.

There is a need for a simple linkage to connect a target cell
to an insoluble support, which do not involve compounds of a
rather unspecified haptene-group, and which is directed to
detection of specific target cells, with a minimum of
unspecific cell association and which render unnecessary a
later cleavage between the insoluble support and the specific
target cell.

Thus the object of the present invention is to detect for
diagnostic purposes specific target cells when used in a blood
and bone marrow without the problem with unspecific binding to
normal cells. It represents a sensitive detection method for a
variety of cell types, such that a high number of cells can be
readily screened in the microscope and the procedure is rapid
and simple. Furthermore, the present method can be used for
isolation of cells for biochemical, biological and
immunological examination, and for studying of specific genes
at the nucleotide or protein level, in addition to culturing
the cells, without the need for cleaving the cell-particles
complex. A further object of the invention is to provide a kit
for performing the method as characterized in the claims.

The intensions of the inventions are obtained by the method and
kit characterized in the enclosed claims.

The method for immunomagnetic detection of target cells in a
mixed cell population and physiological solutions containing
cell populations is suitable for detection, but may also be
used in positive isolation of specific types of both normal
cells and patogenic cells. The method creates a linkage between
a specific target cell and an insoluble support, such as

4

paramagnetic particles , which consists of one or two elements.
The particle is either coated with an anti-cell antibody of
murine or human origin, directed to the specific antigen
determinants in the membranes of the wanted target-cells, or
the particles are coated with a polyclonal anti-mouse or anti-
human antibody capable of binding to the Fc-portions of the
specific anti-cell antibody directed to the antigen
determinants in the target-cell membranes. Instead of using the
polyclonal anti-mouse/anti-human antibody for coating the
particles, a monoclonal rat anti-mouse/anti-human antibody may
be used. This last antibody, due partly to its monoclonal
origin, may provide a more specific binding to the anti-cell
antibody and reduce the risk for possible cross-reactions with
other cells in solutions, such as blood. Furthermore,
incubation of the cell suspension with a mild detergent and/or
second set of antibodies or antibody fragments, prelabeled or
not with fluorescent agents, metallocolloids, radioisotopes,
biotin-complexes or certain enzymes allowing visualization,
will dramatically increase the specificity of the method.

In the following a more detailed disclosure of the method is
presented, using cancer cells as the target-cells for detection
and possible isolation. The method is, however, not limited to
cancer cells and the disclosure shall not limit the method to
this particular field of use, since the method is suitable
within a range of cytological research areas.

In the management of cancer patients, the staging of the
disease with regards to whether it is localized or if
metastatic spread has occurred to other tissues, is of utmost
importance for the choice of therapeutic alternative for the
individual patient. Malignant cells spread by direct invasion
into the surrounding tissue, through the lymphatics or by the
distribution of tumor cells in the blood to distant organs,
including the bone marrow and the central nervous system and
the cerebrospinal fluid.

Detection of metastatic tumor cells has, until recently, relied
on morphological methods using light and electron microscopy on
biopsied tumor specimens, on smears of bone marrow and
peripheral blood, and on slides prepared after
cytosentrifugation of various body fluids. Since the advent of
monoclonal antibodies recognising antigens predominantly
expressed on the surface of different types of malignant cells,
the identification of metastatic cells has, to an increasing
extent, also involved immunocytochemistry and
immunofluorescence. Thus, slides prepared from biopsied tumors
or cytosentrifugates have been treated with monoclonal
antibodies, and the binding of these to the tumor cells is
visualized colorimetrically or by fluorescence. The latter
method requires the use of a fluorescence microscope,
alternatively preparing a cellsuspension an use a flow
cytometer.

The previous methods suffer from limited sensitivity and/or
specificity, and is usually laborious and time consuming, also
requiring a high degree of expertise. Flowcytometric
examiniations also involve expensive equipment.

The morphological methods for the detection of tumor cells in
blood and bone marrow are much less sensitive than methods
involving immunocytochemistry and immunofluorescence (Beiske et
al., Am. J. Pathology 141 (3), September 1992). Also the latter
methods are, however, inadequate in cases where the tumor cells
represent less than 1 % of the total number of nucleated cells.
Flow cytometry may provide better sensitivity than the methods
involving the use of a microscope, but requires the
availability of a high number of cells, and also involves
several technical difficulties. Thus, aggregation of cells may
cause problems, and the method does not provide possibilities
to distinguish between labeled tumor cells and unspecifically
fluorescing normal cells.

The invention allows for a very sensitive detection of, for
example, metastatic tumor cells, since a high number of cells

6

can readily be screened in the microscope and the attached
magnetic beads are easily recognisable. The monoclonal
antibodies used bind with sufficient specificity to, for
example, tumor cells and not to other cells than the target-
cells present in mixed cell suspensions, like blood, bone
marrow, and in other tumor manifestations, such that all cells
with attached beads represent the target-cells. In addition,
the procedure is rapid and simple, and can be performed by any
investigator with access to a conventional microscope.

The novel method involves the binding of monoclonal antibodies,
e.g. of murine or human origin, that specifically recognize
antigens present on tumor cells, and not on the normal cells in
question, or for other purposes to specified subpopulations of
normal cells, to paramagnetic particles, either directly or to
beads first covered with antibodies specifically recognizing
the respective antibodies, or the Fc-portion of IgG antibodies,
that bind to the tumor cells. ████████████████████████████████
be of the IgG or IgM type or being a fragment of ab IgG or IgM.
Examples of used anti-target-cell antibodies may be those
directed against groups of antigen determinants, for example
CD56/NCAM antigen (MOC-1), Cluster 2 epithelial antigen (MOC-
31), Cluster 2 (MW~40kD) antigen (NrLu10) (Myklebust et al. Br.
J. Cancer Suppl. 63, 49-53, 1991), HMW-melanoma-associated
antigen (9.2, 27) (Morgan et al., Hybridoma, 1, 27-36, 1981),
80kD, Sarcoma-associated antigen (TP1 & TP3) (Cancer Res. 48,
5302-5309, 1988), mucin antigens (Diel et al., Breast Cancer
Res. Treatm., 1991), or EGF-receptor antigen (425.3) (Merck),
in addition to the anti-pan-human antibody (Bruland et al.,
unpublished), which is suitable for detecting human cells
among animal cells. The 425.3 antibody is directed towards
antigens in both normal and malignant cells. Antibodies can
furthermore be directed against growth factor receptors, for
example EGF-receptor, PDGF (A and B) receptor, insuline
receptor, insuline-like receptor, transferrin receptor, NGF
and FGF receptors, group of integrins, other adhesion membrane
molecules and MDR proteins in both normal cells and abnormal
cells, and antigens present on subpopulations of normal cells,

in addition to oncogenic products, expressed on the membranes
of normal and malignant cells and on malignant cells alone, for
example Neu/erb B2/HER2. As for the malignant cells, these may
be breast, ovarian and lung carcinoma cells, melanoma, sarcoma,
glioblastoma, cancer cells of the gastrointestinal tract and
the reticuloendothelial system, or the target-cells may be
associated with non-neoplastic diseases, such as
cardiovascular, neurological, pulmonary, autoimmune,
gastrointestial, genitourinary, reticuloendothelial and other
disorders. Furthermore, the malignant cell population may be
located in bone marrow, peripheral blood, come from pleural and
peritoneal effusions and other body fluid compartments, such as
urine, cerebrospinal fluid, semen, lymph or from solid tumors
in normal tissues and organs, for example liver, lymph nodes,
spleen, lung, pancreas, bone tissue, the central nervous
system, prostatic gland, skin and mucous membranes. A more
complete list of the antigen determinants and the corresponding
antibodies or antibody fragments used in the present improved
method is presented in Table 1.

The method comprises attachment of the antibodies directly to
the paramagnetic particles, or the attachment can take place
by attachment to surface-bound antibodies, such as polyclonal
anti-mouse antibodies, monoclonal rat anti-mouse antibodies or
monoclonal anti-human antibodies, specifically recognizing the
Fc-portion of the said individual antibodies. The antibody-
coated paramagnetic beads are then mixed with the suspension of
cells to be examined and incubated for 5-10 min to 2 h,
preferably for 30 min at 0-25°C, preferably at 4°C, under
gentle rotation. The present method may also be performed in a
changed order of steps, in that the free target-cell antibodies
are added to the cell suspension, incubated for 5-10 min to 2h,
preferably 30 min, at 0-20°C, preferably 4°C, under gentle
rotation. The paramagnetic particles, precoated with anti-mouse
or anti-human antibodies are then added to the incubated cell
suspension, as described above, and the resulting suspension
subjected to a further incubation of 5-10 min to 2h, preferably
30 min, at 0-25°C, preferably 4°C under gentle agitation.

8

Samples of the cell suspension are then transferred to a cell
counting device, and the fraction of cells with attached beads
relative to the total number of cells is determined under light
microscopy. The number of antibody-coated beads added to the
cell suspension should be between 0.5-10 times the number of
target cells. When this number is unknown, the amount of coated
beads added should be 1-10 % of the total number of cells.

For specific purposes, and in the cases where the density of
the target-cells is low, for example malignant cells, or the
target-cells represent a very low fraction of the total number
of cells ($\leq$ 1%), the target cells can be positively separated
from non-target cells in a magnetic field. The isolated target
cells, can then be enumerated microscopically and the fraction
of target cells relative to the total number of cells in the
initial cell suspension can be calculated. Moreover, the
target-cells may be characterized for the presence of specific
biochemical and biological features. Of particular importance
will be the use of such cells for studies in molecular biology.
In contrast to the above cited methods of the prior art, the
present method allows studies and growth of the target-cells
without performing a cleavage of the paramagnetic particle-
target cell linkage. For several purposes it is of interest to
examine specific genes in a pure population of target cells at
the DNA, mRNA and protein level, both in tumor biopsies as well
as in tumor cells present in blood, bone marrow and other body
fluids, for example urine, cerebrospinal fluid, semen, lymph,
or from otherwise normal tissues and organs, for example liver,
lymph nodes, spleen, lung, pancreas, bone tissues, central
nervous system, prostatic gland, skin and mucous membranes, and
in other areas of cytological research activity.

With the methods of prior art, signals obtained on Southern,
Northern and Western blots represent the normal cells as well
as the tumor cells in the biopsy. If a single cell suspension
is first prepared from the tumor material, and the tumor cells
are then positively immunomagnetically detected and separated,
any gene studies performed on this material would represent the

target-cells only. This also relates to for example malignant
cells present in mammalian tissues, for example in bone marrow,
peripheral blood, pleural and peritoneal effusions, and other
body fluids, for example urine, cerebrospinal fluid, semen and
lymph. Studies involving polymerase chain reaction (PCR)
methodology will also gain in specificity and reliability when
performed on pure tumor cell populations obtained by the new
method.

The application of the new method steps may differ depending on
type of tissues to be examined.

a)    Tissue from solid or needle tumor biopsies is prepared
mechanically or with mild enzymatic treatment into a single
cell suspension, to which the primary, specific antibodies or
antibody fragments are added directly or after washing the cell
suspension with phosphate buffered saline or culture medium
with or without serum, such as fetal calf serum, bovine, horse,
pig, goat or human serum.

b)    If the material is a sample of pleural or ascitic
effusion, cerebrospinal fluid, urine, lymph or body fluids such
as effusions in the joints of patients with various forms of
arthritis, the specific antibodies or antibody fragments are
either added to the samples directly, or after centrifugation
with or without washings before or after the cells in the
samples are spun down and brought back into suspension.

c)    If the material consists of blood or bone marrow aspirate,
the mononuclear cell fraction is isolated by gradient
sentrifugation on e.g. Lymphoprep before washing, resuspension,
and addition of the appropriate antibodies or antibody
fragments.

The procedure conditions for a) and b) are established, as
exemplified by results obtained in successful experiments as
those described below.

For c) the results have been found to be influenced by a high
number of factors which have been examined in detail. Among
these are antibody concentration, the ratio of the number of
paramagnetic particles versus number of cells, incubation times
and volumes, type of incubation medium, and the pH level. The
particle to mononuclear cell ratio in all experiments should be
in the range of 0.5/1 - 2/1, depending on the binding affinity
of the primary specific antibodies or fragments.

A major problem has been unspecific attachment to normal blood
or bone marrow cells of particles coated with either sheep or
rat anti-mouse antibodies alone, or in addition with the
specific antibodies. Experiments have shown that the unspecific
binding is equally high without the presence of the specific
antibodies, indicating that the problem is not caused by cross-
reactivity of the targeting antibodies to normal cells. The
possibility that the less than optimal specificity could be
caused by ionic binding has been ruled out. Another possibility
was that subpopulations of normal cells of the B-lineage might
adhere to the particle-antibody complexes. However,
immunomagnetic removal of B-cells from the cell suspension
before adding the specific antibodies/antibody-particle
complexes did not improve the specificity of the latter.

The problem with the procedure used on isolated mononuclear
fractions of bone marrow and peripheral bllod, that some non-
target cells might also bind paramagnetic particles, has been
circumvented or overcome. Thus with sheep-anti-mouse antibody
coated particles alone or with specific antibodies the number
of particles unspecifically attached to a low fraction
mononuclear blood or bone marrow cells was reduced from an
average of 10 to about 1 and in paralell the fraction of normal
cells with particles decreased from 1-2% to 0.5-1% or less.

Evidence has been obtained that the problem may be caused by
hydrophobic forces associated with the antibodies bound to the
paramagnetic particles. Methods for reducing this
hydrophobicity is thus claimed. One such method is

preincubation of the antibody-coated particles and the cell suspension with mild detergents in suitable concentrations, for example Tween 20 in concentrations of less than 0.1% for 30 minutes at 4°C. When possible selection of the target cells is warrented, the cell suspension should contain a low concentration of the detergent, e.g. 0.01% of Tween 20. In several experiments this procedure has almost eliminated or dramatically reduced the problem of unspecific binding seen with the mononuclear cell fractions from blood or bone marrow.

The other improvement which, if found warrantied, may be used together with the detergent step as follows:

After incubation of the cell suspension with the primary antibodies or antibody fragments and the antibody-coated paramagnetic particles as described in previously, the cell suspension is incubated with a second set of antibodies or antibody fragments directed against other extracellular or against intracellular determinants of the target cells, with our without pretreatment with cell fixatives such as formaldehyde or alcohols. These antibodies or their fragments should have been prelabeled by fluorescent agents, metallocolloids, radioisotopes, biotin-complexes or enzymes like peroxidase and alkaline phosphatase, allowing visualization by per se known methods in the microscope and/or a suitable counting device.

The target cells will both be visualized with the latter method and have bound particles to their surface, and can thus be enumerated.

To simplify the distinction between non-target and target cells, the cell suspension can before the second visualization step either be subjected to cytospin centrifugation or portions of the suspension are attached to caoted glass slides on which the particle-bound cells will be spread out in a thin layer, facilitating the recognition of the double-"stained" cells.

12

For use in the new procedure, kits will contain for example precoated paramagnetic particles prepared for each monoclonal antibody. In another embodiment the kits contain paramagnetic particles pre-coated with IgG isotype specific anti-mouse or anti-human antibody as one part of it, and different target cell-associated, for example tumor cell, antibodies as another part. In a third embodiment the kit contains paramagnetic particles precoated with specific anti-Fc antibodies, such as polyclonal anti-mouse, or monoclonal rat anti-mouse, or anti-mouse, or anti-human antibodies, capable of binding to the Fc-portion the target-cell associating antibodies, bound to specific anti-target-cell antibodies. In a further embodiment the kit contains other specific antibodies or antibody fragments directed against antigens/receptors within or on the wanted target-cells, where said antibodies or antibody fragments are conjugated to peroxidase, alkaline phosphatase, or other enzymes, together with relevant substrates to such enzymes, or where said antibody or antibody fragment is bound to non-paramagnetic particles with specific colours or with bound enzymes such as peroxidase and alkaline phosphatase.

The present method will in the following be illustrated by model experiments, examples of the usefulness of the new method and examples of practical applications. These examples shall not be regarded as in any way limiting the invention.

Model experiments:

1.    Binding of antibody-bead complexes to tumor cell lines with the new procedure:

To determine antibody concentrations and optimal conditions for the binding of antibody-paramagnetic particle complexes to tumor cells, a large panel of cancer cell lines was used. The paramagnetic beads were bound to the cells, either by coating the specific antibodies to sheep-anti-mouse antibody (SAM)-coated paramagnetic particles, or by first incubating the cells with the specific antibodies, washing, followed by a second

13

incubation with SAM-coated particles. The results of these
experiments are given in Tables 2a and 2b, in which + indicates
binding of several beads to all cells, (+) indicates either a
lower number of beads bound to each cell, or that not all the
tumor cells had beads attached to their surface, whereas -
reflects no binding, and (-) indicates very weak binding.


2.   For detection of tumor cells in the mononuclear fraction
of bone marrow or peripheral blood, model experiments were
performed where specific antibodies and SAM-coated paramagnetic
particles were added either to such mononuclear cells or to a
cell suspension where a different number of cancer cells from
in vitro cultivated cell lines were added to said mononuclear
cells. In some experiments, either the mononuclear cells, or
the malignant cells were prestained with a fluorescent dye, to
be able to distinguish between the two types of cells. In all
experiments, non-binding primary antibodies, and/or sheep-anti-
mouse antibody-coated beads were used separately as controls.

Table 2a

| Antibodies | | Cell lines | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | MCF-7 | SKBR3 | T47D | MDA231 | MDA435 | DU145 | FMEX-1 | LOX |
| | | | | | | | | | · |
| NrLu10 | IgG2b | · | + | + | (+) | (+) | + | | |
| Moc31 | IgG1 | + | + | + | (+) | (+) | + | | |
| Mocl | IgG1 | | | (+) | (+) | + | | | |
| 12H12 | IgG1 | | + | + | | + | + | | |
| 2E11 | IgG3 | + | + | + | | + | + | | |
| 5A6 | IgG1 | | (+) | + | | | | | |
| 5F2 | IgM | | | (+) | | | | | |
| CC3 | IgG2a | - | - | - | | | | - | |
| CC1 | IgM | | | - | | | | (+) | |
| CU18 | IgG1 | - | - | - | | | | | |
| CU46 | IgG1 | (+) | - | - | | | | | |
| 7F11 | IgG1 | - | - | + | | | - | - | - |
| D7 | IgG3 | | | (+) | | | | | |
| E4SF | IgG1? | | + | + | | | (-) | - | 50%+ |
| 425-3 | | | | + | | | | - | + |
| 9.2.27 | | | | | | | | + | + |
| MUC18 | | | - | | | | - | - | - |
| 2g12 | IgG1 | | | | | | | + | |
| 4b7 | IgG1 | | | | | | | + | |
| BM2 (=2F11) | | | | | | | | | |
| BM7 (=7F11) | | | | | | | | | |
| TP-3 | | | | | | | | | |
| TP-1 | | | | | | | | | |
| CEA | | | | | | | | | |
| GINTES | IgG | | | | | | | | |
| 3C9 | IgM | | | | | | | | |
| HH8 | IgM | | | | | | | | |
| 5F4 | IgM | | | | | | | | |
| 3F1 | IgG1 | | | | | | | | |

Table 2b

| Antibodies | | Cell lines | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | PM1 | MA-11 | CRL1435 | CRL1740 | H-146 | Colo205 | 786-0 | WIDR |
| | | | | | | | | | |
| NrLu10 | IgG2b | + | + | + | + | + | + | − | |
| Moc31 | IgG1 | + | + | + | + | + | + | + | + |
| Moc1 | IgG1 | | | | | + | − | | |
| 12H12 | IgG1 | + | + | (+) | | − | − | − | |
| 2E11 | IgG3 | (+) | + | − | + | − | − | − | |
| 5A6 | IgG1 | + | + | | | | | | |
| 5F2 | IgM | | | | | | | | |
| CC3 | IgG2a | | | | | − | | − | |
| CC1 | IgM | | | | | (+) | | − | |
| CU18 | IgG1 | | | | | − | | − | |
| CU46 | IgG1 | | | | | − | | − | |
| 7F11 | IgG1 | (+) | + | − | | − | − | | − |
| ID7 | IgG3 | | | | | − | | | − |
| E4SF | IgG1? | + | + | + | + | − | − | | − |
| 425-3 | | | | | | | | | |
| 9.2.27 | | | | | | | | | |
| MUC18 | | − | | | | | | | |
| 2g12 | IgG1 | | | | | − | | − | |
| 4b7 | IgG1 | | | | | − | | − | |
| BM2 (=2F11) | | + | + | | | | | | |
| BM7 (=7F11) | | + | | | | | | | |
| TP-3 | | | | | | | | | |
| TP-1 | | | | | | | | | |
| CEA | | | | | | | | | |
| GINTES | IgG | | | | | + | | | − |
| 3C9 | IgM | | | | | − | | | − |
| HH8 | IgM | | | | | − | | | − |
| 5F4 | IgM | | | | | − | | | − |
| 3F1 | IgG1 | | | | | − | | | − |

16

In several experiments some unspecific binding to the mononuclear cells was observed, which was found to be unrelated to the nature of the specific antibody, and which was equally pronounced with SAM-coated particles alone. The magnitude of this unspecific binding varied from almost 0 to a level between 0.5-2%. This unspecific binding was almost eliminated by mild treatment with detergent, (Tween 20) performed to reduce the problem of hydrophobic cell interactions.

EXAMPLES OF THE USEFULNESS OF THE NEW PROCEDURE

1.    Detection of micrometastic neoplastic disease in blood and
      marrow

Early and reliable diagnosis of spread of cancer cells to blood and/or bone marrow has become increasingly important for the choice of optimal therapy, possibly curative in many types of cancer, including carcinomas, as described in application Example 1. Similar procedures for malignant melanoma, sarcoma, neuroblastoma and several other cancers have been established or are under development.

2.    Detection of malignant cells in pleural or ascitic
      effusions, and in urine

The nature of such effusions may represent an important diagnostic problem, particularly when a low number of cancer cells are present together with normal reactive or epithelial cells. In several cases a definite diagnosis has been rapidly made with the new method, in cases where conventional cytological examination has been negative or inconclusive. A similar advantage can be found in cases of cancer in the kidneys or in the urinary tract and bladder.

3.  <u>Detection of neoplastic cells in the cerebrospinal fluid</u>

As the systemic treatment of many cancer types have improved,
the frequency of cases with symptom-giving brain metastases
have significantly increased, and in parallell with this, the
necessity for early detection of such spread. With the use of
the new procedure even a low number of malignant cells can
easily be identified, permitting intervention with therapeutic
alternatives at an early stage of intracranial tumor
manifestations.

4.  <u>Diagnosis of cancer in biopsied tissue</u>

When cancer is suspected, and tissue biopsies are obtained by
surgical procedures or by e.g. needle biopsies, a much more
simple and rapid diagnosis can be made with the new method,
used on prepared cell suspensions, compared to conventional
morphological or immunohisto- or cytochemical procedures.

Distinction between several alternative cancers can be made by
the use of the appropriate antibodies.

5.  <u>Identification of prognostic indicators</u>

Since the expression of several membrane molecules have been
shown to correlate with progression of the malignant disease in
several cancers, the present method can be used to identify
prognostic indicators, for example as described in application
Example 2.

6.  <u>Identification of cells indicative of specific diseases or
    of disease progression or state</u>

In various types of rheumatoid diseases (such as rheumatoid
arthritis), as well as in allergic, autoimmune, and
cardiovascular diseases, identification of the systemic or
local presence of specific subpopulations of cells is important

18

for diagnosis and for determining the stage of the disease. Rapid detection of such cell populations with the new method is therefore of considerable diagnostic and therapeutic importance.

7.   Detection of subpopulations of normal cells

For several purposes, it will be important to detect the fraction of a particular subpopulation of normal cells in a population. This applies e.g. to liver biopsies where the identification of cells expressing the biliar epithelial antigen, may be of importance. Similarly, the identification, and possible isolation of specific endothelial cells from a cell suspension prepared from various normal tissues may be warranted.

Several of the cell membrane molecules mentioned in sections 1-6 may also be used as targets for immunotherapy with several types of activiated killer cells or e.g. with immunotoxins. The identification with the new method of expression of such molecules is, therefore, also of value for determining in which cases such types of therapy should be used.

Examples of a practical application of the method:

Example 1
To diagnose spread of cancer cells in blood and/or bone marrow at an early stage, we have used in the new procedure the MOC-31, NrLu10, BM2, BM7, 12H12, and MLuC1 anti-carcinoma antibodies to determine whether or not micrometastatic disease from breast, lung, colorectal, and prostate cancer might be sensitively identified in such body fluids. The successful results with these antibodies have significant clinical implications.

Example 2
The expression of serveral cell membrane molecules have been shown to correlate with progression of the malignant disease in

19

several types of cancer. .e detection of binding of such
antibodies to respective antibodies can therefore be used to
obtain information of high prognostic value. Among such
antigens are a high number of adhesion molecules, carbohydrate
antigens, glycolipids, growth factor receptors and carcinoma
markers listed below. We have, with the new procedure
identified the binding of particle-antibody complexes to CD44-
variants, E-cadherin, Le$^y$, CEA, EGF-r, transferrin receptor,
MUC-1 epitope, LUBCRU-G7 epitope, prostate cancer antigen,
UJ13A epitope, $\beta_2$-microglobulin, HLA-antigens, and apoptosis
receptor.


Example 3

Two litres of pleural diffusion from a patient supposed to
suffer from malignant melanoma was obtained. After
centrifugation, the cells were suspended in a volume of 2 ml of
RPMI with a 10% fetal calf serum, incubated with 9.2.27 anti-
melanoma antibody (10 $\mu$g/ml) at 4$^o$C for 30 min, washed and
again incubated with Dynabeads SAM M450/IgG2A at 4$^o$C for
30 min. The cell suspension was then examined under a
microscope for determining the fraction of cells with
paramagnetic cells attached to their surface. The diagnosis of
malignant melanoma was confirmed, as about 10% of the cells had
a significant number of particles rosettes.


Example 4

Biopsied tissue was obtained from a subcutaneous tumor in a
case with clinical indications of either small cell lung cancer
or a malignant melanoma. A single cell suspension was prepared
from the biopsy, divided in 2 fractions, one incubated with the
9.2.27 anti-melanoma antibody, and the other with MOC-31 anti-
carcinoma antibody (both at 10 $\mu$g/ml). The incubation was
similar to that used in the example above. None of the cells
incubated with the melanoma antibody bound any beads, whereas
all tumor cells incubated with MOC-31 were positive.


Example 5

20

Biopsied tissue from a patient suspected to have malignant
melanoma was examined by preparing single cell suspension,
incubating with 9.2.27 anti-melanoma antibody, and then
following the procedure as above. Most of the cells were
positive with a high number of particle-rosettes attached to
their membranes.


Example 6

A pleural effusion from a breast cancer patient was studied to
examine whether tumor cells could be detected in the fluid. One
litre of the fluid was centrifuged, the cells resuspended, and
in separate vials incubate with each of 3 different anti-
carcinoma antibodies (MOC-31, 2E11, 12H12). After completing
the procedure as in the previous example, it was found that
most of the cells bound to antibody-coated particles in all 3
cases.


Example 7

A bone marrow suspension obtained from a breast cancer patient
was studied to examine whether micrometastic tumor cells could
be present. After the preparation of mononuclear cells, these
were incubated with the same 3 anti-carcinoma antibodies used
in the example above, but in this case the antibodies were
first attached to Dynabeads SAM IgG paramagneteic particles.
After 1 incubation with these directly coated particles, the
cell suspension was examined in the microscope, and a high
number of cells were found positive with a number of particle-
rosettes attached to their membrane.


Similar experiments have been performed in a number of pleural
or ascitic effusion and bone marrow from patients with breast
cancer.


Example 8

T47D human breast carcinoma cells were incubated for varying
lenghts of time with Hoechst fluoresence dye, and the viability
of the labeled cells was checked. Varying numbers of labeled
breast carcinoma cells were then added to $1 \times 10^6$ bone marrow

cells obtained from healthy volunteers. In different
experiments, different concentrations of paramagnetic,
monodisperse particles (Dynabeads P450) coated with individual
anticarcinoma antibodies (NrLu10, MOC31, or 12H12) were added.
After incubation for 30 min on ice, samples of the different
test tubes were examined in a counting chamber under light and
fluorescence microscopy. When the ratio of tumor cells/total
nucleated cells was low, the cell suspension was subjected to a
magnetic field and the cells with particles attached were
isolated before examined in the microscope. It was found that
at an optimal ratio of 1-10 paramagnetic beads per tumor cell
in the cell mixture, all the tumor cells had from 2-15 beads
attached to their surface. The sensitivity of the detection
method was close to one target-cell per $10^4$ nucleated cells.
In control experiments with labeled tumor cells using
antibodies known to have some cross-reativity to normal cells,
this cross-reactivity was confirmed with the antibody-coated
paramagnetic particles. In experiments with beads without
tumor-associated antibody coating, none of the target cells
bound any beads.

Similar experiments have been performed both with other breast
cancer lines and a small cell lung cancer cell line. Similar
sensitivity and specificity were obtained in these experiments.

Example 9
Pleural and ascites fluid from patients with breast cancer and
ovarian carcinoma were sentrifuged, the same coated
paramagnetic particles used in Example 1 were added, incubated
and concentrated in a magnetic field before the suspension was
examined under light microscopy. Typically, cells that had the
clear morphological features of tumor cells had beads attached,
whereas none of the few normal cells bound the antibody-coated
beads. In two cases with pleural effusion, an independent
morphological examination did not reveal the presence of any
tumor cells, whereas a significant number malignant cells were
detected by the use of anibody-coated beads. In some cases,
tumor cells were separated in a magnetic field and transferred

to tissue culture flasks containing growth medium specially
prepared for growing breast cancer cells, in attempts to
establish permanent cell lines from these cultures. In
parallel, cells from the malignant effusions were cultivated
directly without positive selection with magnetic beads. In the
latter cases, no cell line could be established, whereas in
more than 50 % of the cases where positively selected tumor
cells had been used, cell lines were successfully established.


Example 10
In some cases, bone marrow and peripheral blood obtained from
patients with breast cancer were examined with the present
procedure by adding antibody-coated paramagnetic beads,
incubating for 30 min at 4°C and concentrating in a magnetic
field and by examining the suspension under light microscopy.
In both cases binding of the paramagnetic beads to tumor cells,
representing 0,1-1 % of the nucleated cells in the bone marrow
and blood was detected, cells that could not be identified by
any other method.


Example 11
Antibodies against certain growth factor receptors or other
gene products expressed on the surface of specific cell
populations may be used to identify and positively select these
cells. Beads coated with anti-transferrin receptor antibodies,
used in the novel method according to the present invention
were shown to represent a rapid, simple and sensitive method
for identification of cells expressing the transferrin-
receptor.


Example 12
For various purposes isolation of specific populations of
normal cells is warranted. Endothelial cells lining the
capillary or small vessels in normal or tumorous tissue could
be positively selected from cell suspensions prepared from the
relevant tissues. The procedure involved the use of beads
coated with antibody directed against structures expressed on

the endothelial cells, but not on the other normal cells in the
cell mixture.

Example 13
Human cells injected into immunodeficient rodents was shown to
be present in cell suspensions prepared from tumor xenografts
and from various host organs/tissues by employing magnetic
particles coated with an anti-pan human antibody.

24

Table 1

LIST OF RELEVANT ANTIGENS AND EXAMPLES OF ASSOCIATED ANTIGEN-BINDING ANTIBODIES

| ANTIGENS | MONOCLONAL ANTIBODIES |
|---|---|
| **Adhesion molecules** | |
| Fibronectin receptor ($\alpha 5\beta 1$ integrin) | Pierce 36114, BTC 21/22 |
| | Calbiochem 341649 |
| Integrin $\alpha 3\beta 1$ | M-Kiol 2 |
| Vitronectin receptor ($\alpha v\beta 3$ integrin) | TP36.1, BTC 41/42 |
| Integrin $\alpha 2$ | Calbiochem 407277 |
| Integrin $\alpha 3$ | Calbiochem 407278 |
| Integrin $\alpha 4$ | Calbiochem 407279 |
| Integrin $\alpha 5$ | Calbiochem 407280 |
| Integrin $\alpha V$ | Calbiochem 407281 |
| Integrin $\beta 2$ | Calbiochem 407283 |
| Integrin $\beta 4$ | Calbiochem 407284 |
| GpIIbIIIa | 8221 |
| ICAM-I (CD54) | C57-60, CL203.4, RR 1/1[1] |
| VCAM-1 | Genzyme 2137-01 |
| ELAM-1 | Genzyme 2138-01 |
| E-selectin | BBA 8 |
| P-selectin/GMP-140 | BTC 71/72 |
| LFA-3 (CD58) | TS 2/9 |
| CD44 | BM 1441 272, 25.32 |
| CD44-variants | 11.24, 11.31, 11.10 |
| N-CAM(CD56) | MOC-1 |
| H-CAM | BCA9 |
| L-CAM | BM 1441 892 |
| N-CAM | TURA-27 |
| MACAM-1 | NKI-M9 |
| E-cadherin | BTC 111, HECD-1, 6F9 |
| P-cadherin | NCC-CAD-299 |
| Tenascin | BM 1452 193, |
| | Calbiochem 580664 |
| Thrombospondin receptor (CD36) | BM 1441 264 |
| VLA-2 | A1.43 |
| Laminin receptor | |
| HNK-1 epitope | HNK-1 |
| **Carbohydrate antigens** | |
| T-antigen | HH8, HT-8 |
| Tn-antigen | TKH6, BaGs2 |
| Sialyl Tn | TKH-2 |

Table 1 (cont.)

| | |
|---|---|
| Gastrointestinal cancer associated antigen (M,200kD) | CA 19-9 |
| Carcinoma associated antigen | C-50 |
| Le$^y$ | MLuC1, BR96, BR64 |
| di-Le$^x$, tri-Le$^x$ | B3 |
| Dimeric Le$^x$ epitope | NCC-ST-421 |
| H-type 2 | B1 |
| CA15-3 epitope | CA15-3 |
| CEA | I-9, I-14, I-27, II-10, I-46, Calbiochem 250729 |
| | |
| Galb1-4GlcNac (nL4,6,8) | 1B2 |
| H-II | BE2 |
| A type 3 | HH8 |
| Lacto-N-fucopentanose III (CD15) | PM-81 |
| | |
| Glycolipids | |
| GD$_3$ | ME 36.1, R24 |
| GD$_2$ | ME36.1, 3F8, 14.18 |
| Gb$_3$ | 38-13 |
| GM$_3$ | M2590 |
| GM$_2$ | MKI-8, MKI-16, |
| FucGM$_1$ | 1D7, F12 |
| | |
| Growth factor receptors | |
| EGF receptor | 425.3, 2.E9, 225 |
| c-erbB-2 (HER2) | BM 1378 988, 800 E6 |
| PDGFα receptor | Genzyme 1264-00 |
| PDGFβ receptor | Sigma P 7679 |
| Transferrin receptor | OKT 9, D65.30 |
| NGF receptor | BM 1198 637 |
| IL-2 receptor (CD25) | BM 1295 802, BM 1361 937 |
| c-kit | BM 428 616, 14 A3, ID9.3D6 |
| TNF-receptor | GEnzyme 1995-01, PAL-M1 |
| NGF receptor | |
| | |
| Melanoma antigens | |
| High molecular weight antigen (HMW 250.000) | 9.2.27, NrML5, 225.28, 763.74, TP41.2, IND1 |
| | |
| Mw105 melanoma-associated glycoprotein | ME20 |
| 100 kDa antigen (melanoma/carcinoma) | 376.96 |
| gp 113 | MUC 18 |
| p95-100 | PAL-M2 |
| Sp75 | 15.75 |
| gr 100-107 | NKI-bereb |
| MAA | K9.2 |
| M,125kD (gp125) | Mab 436 |
| | |
| Sarcoma antigens | |
| TP-1 and TP-3 epitope | TP-1, TP-3 |

Table 1 (cont.)

$M_r$200kD                                              29-13, 29.2
$M_r$160kD                                              35-16, 30-40


Carcinoma markers
MOC-31 epitope (cluster 2 epithelial antigen)          MOC-31, NrLu10
MUC-1 antigens (such as DF3-epitope (gp290kD))         MUC-1, DF3, BCP-7 to -10
MUC-2 and MUC-3                                        PMH1
LUBCRU-G7 epitope (gp 230kD)                           LUBCRU-G7
Prostate specific antigen                              BM 1276 972
Prostate cancer antigen                                E4-SF
Prostate high molecular antigen $M_r$ > 400kD          PD41
Polymorphic epithelial mucins                          BM-2, BM-7, 12-H-12
Prostate specific membran antigen (Cyt-356)            7E11-C5
Human milk fat globulin                                Immunotech HMFG-1, 27.1
42kD breast carcinoma epitope                          B/9189
$M_r$ > $10^6$ mucin                                   TAG-72, CC-49, CC-83
Ovarian carcinoma OC125 epitope ($m_r$ 750 kD)         OC125
Pancreatic HMW glycoprotein                            DU-PAN-2
Colon antigen Co17-1A ($M_r$37000)                     17-1A
G9-epitope (colon carcinoma)                           G9
Human colonic sulfomucin                               91.9H
$M_r$300kD pancreas antigen                            MUSE11
GA 733.2                                               GA733, KS1.4
TAG 72                                                 B72.3, CC49, CC83
Undefined                                              Oat1, SM1
Pancreatic cancer-associated                           MUSE 11
Pancarcinoma                                           CC49
Prostate adenocarcinoma-antigen                        PD 41
$M_r$150-130kD adenocarcinoma of the lung              AF-10
gp160 lung cancer antigen (Cancer Res. 48, 2768, 1988) anti gp160
$M_r$92kD bladder carcinoma antigen                    3G2-C6
$M_r$600kD bladder carcinoma antigen                   C3
Bladder carcinoma antigen (Cancer Res. 49, 6720, 1989) AN43, BB369
CAR-3 epitop $M_r$ > 400kD                             AR-3
MAM-6 epitope (C15.3)                                  115D8
High molecular ovarian cancer antigen                  OVX1, OVX2
Mucin epitope Ia3                                      Ia3
Hepatocellular carcinoma antigen $M_r$900kD            KM-2
Hepernal epitope (gp43) Hepatocellular carc. ag        Hepema-1
O-linked mucin containing N-glycolylneuraminic acid    3E1.2
$M_r$48kD colorectal carcinoma antigen                 D612
$M_r$71kD breast carcinoma antigen                     BCA 227
16.88 epitope (colorectal carcinoma antigen            16.88
CAK1 (ovarian cancers)                                 K1
Colon specific antigen p                               Mu-1, Mu-2
Lung carcinoma antigen $M_r$350-420kD                  DF-L1, DF-L2

Table 1 (cont.)


gp54 bladder carcinoma antigen                              T16
gp85 bladder carcinoma antigen                              T43
gp25 bladder carcinoma antigen                             T138


Neuroblastoma antigens
Neuroblastoma-associated, such as UJ13A epitope            UJ13A


Glioma antigens
Mel-14 epitope                                             Mel-14


Head and neck cancer antigens
M,18-22kD antigen                                          E48


HLA-antigens
HLA Class 1                                                TP25.99
HLA-A                                                      VF19LL67
HLA-B                                                      H2-149.1
HLA-A2                                                     KS1
HLA-ABC                                                    W6.32
HLA-DR, DQ, DP                                             Q 5/13, B 8.11.2
$\beta_2$-microglobulin                                    NAMB-1


Apoptosis receptor
Apo-1 epitope                                              Apo 1


Various
Plasminogen activator antigens & receptors                Rabbit polyclonal
p-glycoprotein                                             C219, MRK16, JSB-1, 265/F4
cathepsin D                                                CIS-Diagnostici, Italy
biliary epithelial antigen                                 HEA 125
neuroglandular antigen (CD63)                              ME491, NKI-C3, LS62
CD9                                                        TAPA-1, R2, SM23
pan-human cell antigen                                     pan-H

28

CLAIMS

1.   Improved method for detecting specific target cells in
cell suspensions of mixed cell populations and in fluid systems
containing mixed cell populations, and in single cell suspen-
sions prepared from solid tissues, characterized by comprising
the following steps:

1.1.   coating, by a per ce known procedure, paramagnetic
particles or beads with either, a) antibodies, or antibody
fragments directed against membrane structures specifically
expressed on target-cells and not on non-target-cells in the
cell mixture or;
b) antibodies, preferably polyclonal anti-mouse or monoclonal
rat anti-mouse antibodies or anti-human antibodies, capable of
binding to the Fc-portions of the said antibodies, directed
against the membrane structures; and

1.2.1. mixing the target-cell-associating antibodies (murine or
human) which is attached to the said particles or beads, or
attached to the beads pre-coated with anti-mouse or antihuman
antibodies recognizing the Fc-portions of the target-
associating antibodies, with the cell suspension containing the
target-cells, or,

1.2.2. mixing free target-cell-associating antibodies with the
cell suspension containing the target cells and incubate this
mixture for 5-10 min to 2 h, preferably 30 min, at a
temperature between 0°C and 20°C, preferably 4°C under gentle
rotation, and;

1.3.   incubating the mixture of the cell suspension and
target-associating antibodies attached to paramagnetic
particles or beads (1.2.1), or paramagnetic particles or beads,
precoated with anti-mouse or anti-human antibodies recognizing
the Fc-portion of the target-associating antibodies, to the
mixture of incubated free target associating antibody and cell
suspension (1.2.2.), and incubating, for 5-10 min to 2 h,

29

preferably 30 min, at a temperature between 0°C and 25°C,
preferably 4°C, under gentle rotation, and;

1.4.1. if the target cell population is contained in blood or
bone marrow aspirates the hydrophobic forces associated with
antibody-coated particles are reduced by pre-incubating the
antibody-coated particles and the cell suspension with mild
detergents in suitable concentrations, e.g. Tween 20 in
concentrations less than 0.1% for 30 min at 4°C, and/or

1.4.2. by incubating the cell suspensions, untreated or
pretreated with formalin, alcohol or other fixatives, with
other antibodies or antibody fragments binding to extracellular
or intracellular molecules present in the target cells and the
antibodies used are labeled in advance by peroxidase, alkaline
phosphatase, or other enzymes permitting visualization of the
binding by addition and incubation with relevant substrates, or

1.4.3. the antibody fragments are biotinylated and the binding
visualized when adding the incubating with avidin complexed to
peroxidase, alkaline phosphatase, or other enzymes, with
addition and incubation with relevant substrates, or

1.5.1. subjecting the incubated paramagnetic particle-
antibodies-cell mixture (1.3) to a magnetic field if the
density of target-cells is low, or if the ratio of target
cell/total cells in the cell mixture is low ($\leq$ 1 %) and then
examining and counting the stained or unstained particle-
target-cell complexes in the cell suspension, using a
micropscope and/or a suitable cell/particle counting device,
or,

1.5.2. examining and counting the target-cells in the incubated
mixture of paramagnetic particles, antibodies and cell mixture
(1.3), or in the case when the antibodies or antibody fragments
are conjugated to non-paramagnetic particles that can be
visualized directly because of colour or through enzymatic
activation, using a microscope and/or a suitable cell/particle

30

counting device if the ratio of target-cells/total cells in
the cell suspension is adequate (> 1 %).


2.    Method according to claim 1, characterized by directing
the antibody or fragments thereof against the antigens in
normal, living cells, such as liver hepatocytes, Kupffer cells
and endothelial cells type 1 and 2 and Clara cells of the lung,
endothelial cells of specific organs, pancreatic exocrine and
endocrine cells, kidney tubule cells, bladder epithelial cells,
brain glial and ependymal cells, bladder and prostate
epithelial cells, ciliated cells of airways, different
subpopulations of mucosal cells in the gastrointestinal tract,
pituitary cells, and other endocrine cells in various hormone-
producing organs.


3.    Method according to one of the preceding claims,
characterized by using as the said target-cell antibody an
antibody which is reactive with antigens present on
subpopulations of normal cells and oncogenic products expressed
on the membrane of normal tissue cells.


4.    Method according to one of the preceding claims,
characterized by using as the said positive selecting antibody,
an antibody which is directed against growth factor receptors
on the membrane of normal cells, for example the EGF-receptor,
PDGF (A and B) receptor, insuline receptors, insuline-like
receptors transferrin receptor, NGF and FGF receptors.


5.    Method according to one of the preceding claims,
characterized by using an antibody directed against the group
of integrins and other adhesion membrane molecules, and MDR
proteins in normal cells.


6.    Method according to one of the preceding claims,
characterized by directing the antibody or fragments thereof
against antigen or receptors in cells with abnormal
developmental patterns, preferably such as primary and
metastatic cancer cells.

31

7. Method according to one of the preceding claims, characterized by using as the said target-cell associating antibodies, antibodies of the IgG isotype, or $F(ab')_2$ or $F(ab)$ fragments, or IgM, or fragments of IgM.

8. Method according to one of the preceding claims, characterized by preparing the mentioned cell suspension from mixed cell populations comprising mammalian tissues, for examples human bone marrow and peripheral blood, from pleural and peritoneal effusions, other body fluids, for example urine, cerebrospinal fluid, semen, lymph, or from solid tumors in normal tissues and organs, for example liver, lymphatic nodes, spleen, lung, pancreas, bone tissue, central nervous system, prostatic gland, skin and mucous membranes.

9. Method according to one of the preceding claims, characterized by that the antibody or antibody fragments is directed against groups of antigen determinants, such as those listed in the Table 1 of the specification.

10. Method according to one of the preceding claims, characterized by using as the said target-cell antibody an antibody or antibody fragment which is directed against growth factor receptors and oncogene products expressed on the membrane of malignant cells, for example insuline receptors, insuline-like receptors and FGF receptors in addition to those listed in Table 1 of the specification.

11. Method according to one of the preceding claims, characterized by using an antibody or antibody fragment directed against the group of integrins, other adhesion membrane molecules and MDR proteins in abnormal cells as listed in Table 1.

12. Method according to one of the preceding claims, characterized in that the used antibodies, antibody fragments

32

or combinations of these are directed to the antigen
determinants as listed in Table 1 of the specification.


13.   Method according to one of the preceding claims,
characterized by using as the said antibody an antibody which
is reactive with antigens present on abnormal cells, for
example breast, ovarian and lung carcinoma cells, melanoma,
sarcoma, glioblastoma and cancer cells of the gastrointestinal
and genitourinary tract, and of the reticuloendothelial system,
and/or target-cells associated with non-neoplastic diseases,
such as cardiovascular, neurological, pulmonary, autoimmune
gastrointestinal, genitourinary, reticuloendothelial and other
disorders.


14.   Use of the detection method according to one of the
preceding claims, for isolation of target-cells, whereby the
complex of cells and the paramagnetic particles are exposed to
a magnetic field and the resulting magnetically aggregated
cells are further subjected to biological, biochemical and
immunological examinations, including also characterisation of
specific genes at the DNA, mRNA and protein level, including
polymerase chain reaction (PCR) and reverse transcriptase PCR.


15.   Use of the method for detection of specific target-cells
according to one of the preceding claims, whereby it is
established in vitro cell cultures from the separated
paramagnetic particle-target-cell-complexes, and/or for
inoculation into immunodeficient animals, preferably to
establish human tumor xenografts in the said animals.


16.   Kit for performing the method according to one of the
preceding claims, characterized by that it comprises;
1, specific antibodies or antibody fragments directed to the
antigen receptors on the wanted target-cells, where said
antibody or antibody fragment is bound or can be bound to
included paramagnetic particles, without removing their
antigen-binding ability, and/or

33

2,     paramagnetic particles precoated with specific anti-Fc
antibodies, preferably polyclonal anti-mouse, or monoclonal rat
anti-mouse, or anti-human antibodies, capable of binding to the
Fc portions of the target-cell associating antibodies, and
specific free target-cell antibodies, and/or

3,     paramagnetic particles precoated with specific anti-Fc
antibodies, preferably polyclonal anti-mouse, or monoclonal rat
anti-mouse, or anti-human antibodies, capable of binding to the
Fc-portions of the target-cell associating antibodies, bound to
specific anti-target-cell antibodies, and/or

4,     other specific antibodies or antibody fragments directed
against antigens/receptors within or on the wanted target
cells, where said antibodies or antibody fragments are
conjugated to biotin, peroxidase, alkaline phosphatase, or
other enzymes, or where said antibodies or antibody fragments
are bound to non-paramagnetic particles with specific colours
or with bound enzymes such as peroxidase and alkaline
phosphatase.

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# INTERNATIONAL SEARCH REPO

International application No.

PCT/NO 93/00136

## A. CLASSIFICATION OF SUBJECT MATTER

IPC5: G01N 33/53, C12Q 1/00, C12N 5/00
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOTECHNOLOGY

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO, A1, 9204961 (IMMUNICON CORPORATION), 2 April 1992 (02.04.92), see examples 2-4 and in particular col. 14, lines 62-68 | 16 |
| Y | | 1-16 |
| Y | WO, A1, 9109938 (HOLMES MICHAEL JOHNET AL), 11 July 1991 (11.07.91), see the examples | 1-16 |
| X | | 16 |
| X | WO, A1, 9101368 (HOLMES MICHAEL JOHN ET AL), 7 February 1991 (07.02.91) | 16 |

| X | Further documents are listed in the continuation of Box C. | X | See patent family annex. |

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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| Y | EP, A2, 0403960 (BEHRINGWERKE AKTIENGESELLSCHAFT), 27 December 1990 (27.12.90) | 1-16 |

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